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Delineating the immune landscape of Kaposi Sarcoma using multiplexed immunofluorescence at single-cell resolution

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OBJECTIVES/GOALS: KSHV is well documented to infect and establish latency in endothelial cells; however, infected cell types in the Kaposi Sarcoma (KS) tumor microenvironment are not well defined. Here, we present the first application of the Lunaphore COMETTM multiplexed immunofluorescence platform for spatial proteomic analyses of KS lesions. METHODS/STUDY POPULATION: Custom antibody panels were validated and optimized for characterization of the TME components, as compared to marginal structures. The Ab panels supported the detection, quantification, and comparison of >15 cell types in regions of interest in the KS tissues in a single automated run. For data analysis, we developed a rule-based decision tree for accurate cell classification and used the resulting cell populations to perform a nearest-neighbor analysis. We assessed spatial proximity among cell types located within, adjacent to, or distal from the areas with a large number of cells expressing KSHV LANA. RESULTS/ANTICIPATED RESULTS: The highest KSHV load was detected in CD34+ cells with or without CD31, consistent with an endothelial progenitor phenotype (EPCs). We also defined 12 cell types with KSHV-LANA detected in <1% of each cell type. Among them, tumor-infiltrating CTLs were proximal to or overlapping with infected EPCs, suggesting they have cytolytic activities. In addition, we observed areas with T-cells detected at high frequency near normal vascular endothelial cells (VECs), indicating immune cell trafficking toward the lesion in these marginal areas. While supporting previous findings that T-cells are not infected by KSHV, our data also revealed areas having high viral Ag load and abnormal vasculature, versus surrounding areas with much lower Ag load, normal vasculature, and robust immune infiltration. DISCUSSION/SIGNIFICANCE OF IMPACT: Our approach not only demonstrated its ability to characterize the KS TME at a previously unapproachable level of complexity but has also revealed gaps in lineage assignments in substantial proportions of the tumor, which will be subjected to further characterization.

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Social participation and cognitive functioning after traumatic brain injury

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OBJECTIVES/GOALS: The goal of this project is to integrate human and animal data to explore the relationship between social participation and cognitive functioning after traumatic brain injury (TBI). Further, we aim to determine whether reduced social participation is not only a consequence of TBI but a potential driver of post-injury cognitive deficits. METHODS/STUDY POPULATION: This study uses a translational framework to examine how social participation influences cognitive outcomes after traumatic brain injury (TBI) using complementary human and animal models. Longitudinal TBIMS data will be analyzed to assess associations between social participation (PART-O: productivity, out and about, social relations) and cognition (BTACT: memory, fluency, reasoning, processing speed) using linear regression (potentially a Random Intercept Cross-Lagged Panel Model to capture bidirectional effects over time). Further, mice with mild, moderate, or severe TBI will be housed in isolated, standard, or enriched social environments. Post-injury cognitive performance (NOR, Y-maze) and neuronal proliferation and survival in the prefrontal cortex and hippocampus will be evaluated. RESULTS/ANTICIPATED RESULTS: We anticipate that both humans and animals with greater social participation will demonstrate superior cognitive performance over time. In the animal model, we further expect that mice housed in social or enriched environments will exhibit reduced cell death and enhanced neuronal survival in the prefrontal cortex and hippocampus compared to socially isolated counterparts. DISCUSSION/SIGNIFICANCE OF IMPACT: Together, these findings may inform future rehabilitation strategies that integrate social participation as a core component of cognitive recovery supporting more personalized and multidisciplinary approaches to chronic TBI care.

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Enteric colonization with fluoroquinolone-resistant Enterobacterales is associated with increased Enterobacterales and Bacteroidales in hematopoietic cell transplant recipients prior to transplantation

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OBJECTIVES/GOALS: Our objectives were to determine enteric microbiome characteristics in fluoroquinolone-resistant Enterobacterales (FQRE)-colonized and non-FQRE-colonized hematopoietic cell transplant (HCT recipients, and to understand the enteric microbiome-specific factors associated with the development of FQRE BSI in FQRE-colonized HCT recipients. METHODS/STUDY POPULATION: This was a prospective cohort study of patients undergoing HCT at Weill Cornell Medicine from November 2016 to August 2019. All participants received levofloxacin prophylaxis during post-HCT neutropenia. Stool samples were collected prior to levofloxacin prophylaxis initiation and during the week after HCT. Our overall study population consisted of 26 FQRE-colonized HCT recipients and 69 non-FQRE-colonized HCT

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recipients. Samples underwent selective quantitative culture for FQRE, as well as 16S rRNA sequencing. Raw sequence data were processed and aligned to a custom SILVA database. Analysis of 16S rRNA sequencing was conducted in R using phyloseq. RESULTS/ANTICIPATED RESULTS: We saw significant differences in microbiome composition in comparing pre-HCT samples from FQRE-colonized HCT recipients to non-FQRE-colonized HCT recipients, including significantly higher relative abundances of Bacteroidales and Enterobacterales in colonized recipients. MaAslin2 demonstrated significantly differential abundance of Bacteroides and Escherichia-Shigella ASVs. We observed a significant decrease in Enterobacterales relative abundance in non-FQRE-colonized recipients after HCT, but no such change in FQRE-colonized recipients. FQRE-colonized recipients who developed FQRE BSI had a lower relative abundance of Bacteroidales as compared to those who did not. No significant difference in FQRE colonization density as measured by quantitative FQRE stool culture was observed. DISCUSSION/SIGNIFICANCE OF IMPACT: FQRE-colonized HCT recipients represent a unique subgroup of HCT recipients. Colonized HCT recipients with FQRE BSI had lower relative abundance of Bacteroidales compared to those without BSI. Further work is needed to determine the mechanism behind this association and to further characterize the microbiome in this subgroup of HCT recipients.

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Neuropathological hallmarks during the chronic phase of ischemic stroke in mice and humans*

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OBJECTIVES/GOALS: Improvements in acute stroke treatment, including critical care management, have increased survival rates post-stroke. However, many stroke survivors have significant neurological deficits, and stroke remains a leading cause of long-term disability. We aim to study the chronic progression and long-term sequelae of ischemic stroke pathology. METHODS/STUDY POPULATION: We examined long-term neurobehavioral recovery and progressive neuropathology of young (3 months) and middle-aged (14 months) adult male and female C57Bl/6 mice at 1, 3, and 6 months after a 60-minute transient middle cerebral artery occlusion (MCAO) or sham surgery, as well as histopathological analysis of post-mortem brain samples from patients with a chronic infarct. Behavioral examination included the tail suspension test, the novel object recognition test (NORT), and the fear conditioning test (FCT). Molecular and pathological analyses included bulk transcriptomic screening, immunohistochemistry (IHC), flow cytometry, and atrophy analyses via MRI and microCT scanning. RESULTS/ANTICIPATED RESULTS: In mice, depression-like behavior persisted for 6 months post-stroke ($p<0.0001$), while cognitive function progressively worsened from 3 to 6 months post-stroke as seen by NORT (6 months, $p=0.0063$). Memory retention deficits at 6 months were seen in the FCT ($p=0.0084$). Significant brain atrophy was evident by MicroCT and MRI at 2 and 6 months post-stroke. IHC showed significant demyelination (mouse $p=0.0361$; human $p=0.0001$) and increased microglial ($p=0.0059$) and astrocyte activation. TUNEL co-labeling with a neuronal marker showed neuronal apoptosis in human samples of chronic stroke. Disease-associated microglia (DAM) displayed senescent-like phenotypes. Nanostring showed neuroinflammatory pathway upregulation up to 6 months PS. Human stroke brains had more amyloid plaque ($p=0.06$). DISCUSSION/SIGNIFICANCE OF IMPACT: Cellular senescence and chronic neurodegenerative signatures likely result in poor cognitive function that may progressively worsen after stroke. Studying this neurodegenerative etiology in the long-term post-stroke may offer viable targets for delayed stroke treatment, which would fill a severely unmet need for stroke survivors.

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Clinical measures of tibial bone force in runners

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OBJECTIVES/GOALS: Tibial bone stress injuries are running injuries that have a high risk of recurrence. Tibial load contributes to the development of bone stress injuries; however, this variable is clinically inaccessible. The objective of this study is to establish a relationship between tibial load during running and clinical tests to support safe return to running. METHODS/STUDY POPULATION: Thirty-two healthy recreational runners (13 F, 19 M, age 23.6 y, height 1.77 m, mass 73.71 kg, weekly volume 16.91 miles) ran at a self-selected pace on an instrumented treadmill for 5 minutes, while a 7-camera motion capture system collected whole body kinematics. Inverse dynamics were used to calculate ankle joint reaction forces and moments which were then used in a musculoskeletal model to calculate tibial axial force. Single leg heel-raise endurance, hand-held dynamometry ankle strength, and Y-balance tests were also assessed. Linear mixed models will be used to determine relationships between tibial force during running and strength and balance measures. RESULTS/ANTICIPATED RESULTS: Data analysis is ongoing. Based on preliminary data, it appears that peak tibial bone force during running may demonstrate a relationship with lower extremity strength and balance. Clustering participants into "high" and "low" tibial force production during running may help identify specific ranges of strength and balance assessment outcomes that are appropriate for clinical goals. DISCUSSION/SIGNIFICANCE OF IMPACT: The results of this study will attempt to establish relationships between tibial bone loads during running and strength and balance assessments that are clinically feasible. Results have potential to directly impact clinical practice by providing objective measures that may be used as surrogates for tibial bone force in a clinical setting.

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